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Omics studies for Comprehensive Understanding of IgA Nephropathy. State-of-the-Art and Future Directions

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Summary

IgA Nephropathy (IgAN) is the most common worldwide primary glomerulonephritis with a strong autoimmune component . The disease shows variability in both clinical phenotypes and endpoints, and can be potentially subdivided into more homogenous subtypes through the identification of specific molecular biomarkers. This review focuses on the role of -omics in driving the identification of potential molecular subtypes of the disease through the integration of multi-level data from genomics, transcriptomics, epigenomics, proteomics and metabolomics. First, the identification of molecular biomarkers, including mapping of the full spectrum of common and rare IgAN risk alleles, could permit a more precise stratification of IgAN patients. Second, the analysis of transcriptomic patterns and their modulation by epigenetic factors like miRNAs have the potential to increase our understanding in the pathogenic mechanisms of the disease. Third, the specificity of urinary proteomic and metabolomic signatures and the understanding of their functional relevance may contribute to the development of new non-invasive biomarkers for a better molecular characterization of the renal damage and its follow-up. All these approaches can give information for targeted therapeutic decisions and will support novel clinical decision making. In conclusion, we offer a framework of omic studies and outline barriers and potential solutions that should be used for improving the diagnosis and treatment of the disease. The ongoing decade is exploiting novel high-throughput molecular technologies and computational analyses for improving the diagnosis (precision nephrology) and treatment (personalised therapy) of the IgAN subtypes.

Key words

IgA nephropathy, genomics, epigenomics, transcriptomics, proteomics, metabolomics,

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Introduction

IgA Nephropathy (IgAN) is the most common primary glomerulonephritis worldwide [1, 2] that develops mainly in the 2nd and 3rd decade of life and 40% of IgAN cases reach end-stage kidney disease (ESKD) after 20 years from the biopsy-proven diagnosis implying a great socio-economical burden.[3, 4] In this review, we describe the results of omics studies carried out in IgAN patients. We move from genomic studies focusing on common and rare variants linked to the disease, to transcriptomics carried out on circulating immune cells and kidney biopsy specimens and then to epigenomics, proteomics and metabolomics . Finally, we suggest an integrative approach for developing biological networks and identify potential diagnostic biomarkers for Precision Nephrology and Personalized Therapy.

1.1 GENOMICS

Two main genome wide genetic approaches have been used in the study of this complex disease: Genome Wide Association Studies (GWAS) and Next Generation Sequencing (NGS).

1.1.1 Common Variants

The GWAS conducted on IgAN have identified several common variants that clearly show a strong participation of the *Human Leukocyte Antigen* (HLA) system, genes involved in innate immunity, and other loci summarized in Supplementary Table 1. Seven Single-Nucleotide Polymorphisms (SNPs) identified through GWAS were used for constructing a genetic risk score, which explained 4.7% of overall IgAN risk.[5] This score was integrated with other 8 recently associated SNPs,[6] but it cumulatively explains only 5.37% of disease risk (http://www.columbiamedicine.org/divisions/gharavi/calc_genetic.php). In summary, IgAN risk loci identified through GWAS i) are common to other inflammatory and immune-mediated diseases, ii) explain a proportion of the disease risk worldwide, iii) contribute to the geographic variation in disease prevalence, iv) confirm the polygenic and multiple-susceptibility-gene nature of IgAN. Future studies are needed to evaluate if these GWAS signals are effectively generated by causative common variants or due to rare variants in linkage disequilibrium with the common ones. Direct sequencing of the associated regions may lead to a finer mapping of these disease-related loci, as seen for example in chronic obstructive pulmonary disease. [7]

Very recently two quantitative GWAS have identified two loci, in *core 1*, *β1-3-galactosyltransferase-1* (*C1GALT1*) and *C1GALT1 Specific Chaperone 1* (*C1GALT1C1-cosmc*)[8, 9] providing novel insights into the

genetic regulation of O-glycosylation and providing evidence that also common genetic variations can influence O-glycosylation.

Copy Number Variants (CNVs) in IgAN have been reported in complement Factor H Related proteins(*CFHR1*, *CFHR3*) [10, 11] and have been associated with decreased IgAN risk (supplementary Table 2). CNVs were also found enriched in the linked 17q12-22 locus [12, 13] and in the *Toll Like Receptor 9 (TLR9) gene* where its potential role in disease progression was confirmed.[13] A lower copy number of three variants within the defensin highly variable locus correlated with renal dysfunction, increased serum IgA1 and Gd-IgA1.[14, 15]

1.1.2 Rare variants

Rare variants, usually found in the frequency range between 0.1% and 1%, are more likely to have a stronger effect in complex diseases compared to common variants. Three A-Whole Exome Sequencing (WES) studies have been performed on IgAN (Supplementary Table 3). ~~y performed in Chinese with familial IgAN~~ The first study evidenced seven common co-segregating deleterious variants within four genes (*CARD8*, *DEFA4*; *MYCT1*, *ZNF543*).[16] ~~Another~~ The second WES study performed on two affected individuals and an unaffected familial control from a large Sicilian family with multiple affected individuals,⁴⁷ identified a novel missense variant in the *Sprouty RTK Signaling Antagonist 2 (SPRY2)* gene that segregated in all IgAN affected individuals.[17] Functional analysis of the variant in B lymphoblastoid cell lines from affected members linked the *SPRY2* mutation showed the inhibition of the MAPK/ERK pathway. Recently, a combined linkage analysis and exome sequencing methodology has been successfully applied for pinpointing specific causative variants involved in familial goiter.[18] A similar procedure has been carried out by Cox et al[19] in the last IgAN-IgAN study and their results support a polygenic and a multiple-susceptibility-gene model for familial IgAN.[19] Evident connections with previous gene expression studies were found, but further studies are needed to understand whether these variants can effectively disrupt gene function and validate their causality in contributing to IgAN phenotype. Furthermore, these studies must be replicated in other independent cohorts of IgAN patients to confirm the validity of the results.

To date several efforts have been made to identify susceptibility variants, but NGS data for the identification of rare variants within IgAN GWAS areas has never been done. This strategy has been recently applied in the study of age-related macular degeneration in which rare causative coding variants were pinpointed within known associated genetic loci and could represent an innovative and promising approach for IgAN.[20] Association testing deployed in parallel or in combination to familial linkage could represent another innovative strategy for the identification and characterization of a full range of disease-susceptibility variants [21, 22] as seen in hearing impairment and cardiomyopathy .[21-24]

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1.2 TRANSCRIPTOMICS

Transcriptome is considered the gene signature leading to a phenotype that is possibly influenced by genetic determinants. Specific gene expression patterns have been found in IgAN patients' blood cells. (Supplementary Table 4). An aberrant modulation of genes belonging to the WNT-β-catenin and PI3K/AKT pathways was found in peripheral blood leukocytes (PBL) IgAN patients.[25] Monocytes were principally involved in the altered WNT signaling pathway and an expansion of the non-classical CD16+ monocyte subset characterized by an enhanced apoptotic potential was demonstrated.[26] Moreover, transcriptomics was used to analyze gross hematuria episodes in concomitance to mucosal infections,^[26] an important time point for IgAN.[27, 28] Differently regulated genes during the gross hematuria episode were principally involved in interferon signaling and antigen presentation.[28] This network showed an up-regulation of genes involved in the immune-proteasome pathway and based on a series of additional experimental approaches, *C-X3-C Motif Chemokine Receptor 1 (CX3CR1)* and its ligand fractalkine were found to promote macroscopic hematuria in IgAN patients.^[27]— An important limitation of all these experimental approaches is that gene expression data have not been integrated with genome wide genotype data. This integration could be useful for the identification of expression quantitative trait loci (eQTL) giving a direct genetic explanation of aberrant gene expression data, useful for translational medicine.[29]

Most transcriptomic findings have been obtained from complex starting material i.e whole blood, which is made up of different cell lineages. Future studies should propend towards the use of cell sorting technologies for analyzing the transcriptomic profile of specific cell populations or single cell analysis may give novel insights into health and disease status.[30] These novel technological approaches may help to obtain cell-lineage specific expression data from IgA1 secreting cells, giving important insights into the pathogenic mechanisms involved in the disease.

The renal damage in IgAN is caused by mesangial deposition of polymeric IgA1 and or IgA1-IgG immune complexes at glomerular level leading to oversynthesis of extracellular matrix (ECM). Since alterations in mRNA levels could precede the histological damage, transcriptomics in kidney tissue might identify gene expression profiles involved in the development and progression of renal damage.

Investigators focused their attention on the gene expression of isolated glomeruli or tubulointerstitial tissue from IgAN patients' kidney biopsies.^[31, 32] They observed an increased expression of some proteoglycans directly involved in renal damage and suggested their potential role as prognostic

biomarkers and highlighted an 11 transcript proteinuria signature in the tubulo-interstitial compartement. [31, 32] Differently expressed genes in microdissected glomeruli with endocapillary proliferation were involved in innate immune response, classical complement pathway activation and matrix turnover. [33] ~~(supplementary Table 5)~~ Interestingly, the in-silico study demonstrated that the aberrantly expressed genes characterizing endocapillary proliferation were responsive to the combined corticosteroid-resveratrol therapy. [33] ~~Studies on the isolated tubulointerstitial compartment showed a reduced expression of VEGF and an enhanced expression of Decorin. The latter correlated positively with proteinuria [31] and was included in an 11 transcript proteinuria signature [32].~~ A subsequent study demonstrated that altered expressed genes were responsive to some organic substances such as doxorubicin and thapsigargin [34] (see supplementary Table 5).

Data from these studies indicate that (i) glomerular and tubulointerstitial gene expressions are independent; (ii) some genes involved in specific pathways are responsive to drugs. Gene expression data from isolated glomeruli or tubules are not correlated to the extent of renal damage but it's a sterile description of up and down-regulated genes. Future studies should be performed on the entire tissue as whole since kidney disease involves all renal compartments globally and simultaneously. In particular, gene expression studies will need to be performed on well characterized kidney biopsy specimens that have been accurately scored with the new reproducible MEST-C classification system. [35] In this way specific gene expression changes that characterize active renal lesions (E and C) that are more responsive to immunosuppressive therapy compared to chronic lesions (S and T) will be identified. Furthermore, differences in gene expression linked to the degree of histologic renal damage have yet to be evaluated. This information is important because glomerular lesions are always associated with tubulointerstitial damage during the progression of renal damage.

The emerging transcriptomic methodology not deployed in the study of IgAN is RNAseq. This methodology has brought a significant qualitative and quantitative improvement to transcriptome analysis, offering an unprecedented level of resolution able to detect genes expressed at low levels, splice isoforms and novel exons/genes. [36] In addition, allele-specific expression, RNA editing and fusion transcripts represent some of the information that do not emerge from hybridization-based platforms and may be crucial in complex diseases. [37]

1.3 EPIGENOMICS

To date, studies on DNA methylation in IgAN are principally two. (Supplementary Table 6). The first study showed that the gene expression of *Cosmc*, whose activity is closely related to aberrantly glycosylated IgA1,

could be regulated by DNA methylation in lymphocytes of children with IgAN.[38] Limitations of this study, in addition to the small sample size, are the lack of strong validation, functional assays and DNA methylation analysis using a mixed cell population peripheral blood mononuclear cells (PBMCs). The second study showed that the two hypomethylated DNA regions contained the promoters for *Dual Specificity Phosphatase 3 (DUSP3)* and *Tripartite Motif Containing 27 (TRIM27)* and the most extensively hypermethylated region on chromosome 5 contained the *Vault RNA 2-1 (VTRNA2-1)* gene promoter, known as the precursor of miR-886. These aberrantly methylated DNA regions were found to induce a T helper cell imbalance towards the Th1 subtype. Even if the sample size is not very large, this study can be considered confident due to the many validation experiments and functional assays.

MicroRNAs (miRNAs) are another epigenetic component that can modulate gene expression in tissue and biological fluids. [Three different genome-wide miRNA gene expression studies on IgAN patients' blood cells have been performed \(see Supplementary table 7\).](#) ~~A global miRNA expression study performed on IgAN patients PBMCs~~[The first study](#) led to the identification of two [aberrantly expressed](#) miRNAs (let-7b and miR-148b) that regulate the gene expression of two key enzymes involved in the sequential O-glycosylation process of the IgA1 molecule (*GALNT2*, *C1GALT1*).[39] These results were validated biologically with transient transfection experiments and demonstrated that the abnormal miRNA-based regulatory mechanism influences the O-glycosylation process determining the aberrant glycosylation of IgA1, main characteristic of IgAN. [39, 40] These findings could be exploited for therapeutic use because the inhibition of these upregulated miRNAs could normalize the IgA1 O-glycosylation process.

Recently, the miRNA expression profiling of B cells from IgAN patients [41] demonstrated an upregulation of miR-374b that is able to determine B cell proliferation and aberrant IgA1 glycosylation by targeting *C1GALT1C1-Cosmc* and *Phosphatase and Tensin Homolog (PTEN)*; [41] the latter has already been found aberrantly modulated in IgAN.[25] Anti-miR blockers could be exploited for new therapeutic approaches and are being tested in ex-vivo clinical studies on myocardial infarction,[42] neoplasms[43] and in phase III clinical trials for hepatitis C virus infection.[41-44]

While the majority of miRNAs are found within the cell, a handful of circulating miRNAs, released from blood cells, have also been detected in various body fluids. In a multicenter international retrospective study, let-7b and miR-148b levels were measured in serum samples of Caucasian and Asian IgAN patient and their combined value was found as a significant predictor of the disease status, showing a good sensitivity and specificity and discriminating IgAN patients from other primary glomerulonephritides. [45]. This combined biomarker seems to be a novel, specific and non-invasive indicator to test the probability of being affected by IgAN, mainly in patients' relatives with persistent urinary abnormalities.

In the last years, several groups studied the role of miRNAs in kidney tissue. A cluster of miRNAs has been observed in the human kidney tissue with a different distribution in renal cortex and medulla of rat kidney. To date, few studies have been carried out on IgAN renal biopsies (see Supplementary table 8). Abnormal miRNA expression patterns in renal biopsies correlated with glomerular sclerosis and interstitial fibrosis. Various miRNAs have been found to be involved in the progression of renal damage or in the pro-fibrotic processes in IgAN through E-cadherin and the epithelial to mesenchymal transition process. Recently, miRNAs have also been found to have a role in the activation of mesangial cells by secretory IgA (SIgA) from IgAN patients.[43] In conclusion, regarding the use of miRNAs as biomarkers in the blood and kidney, many studies have been conducted in retrospective cohorts but prospective studies are required to confirm their clinical utility and diagnostic value in asymptomatic individuals. In addition, several miRNAs have been identified as targets for the treatment but the use of miRNA modulators in pre-clinical settings is still missing.

1.4 PROTEOMICS

Urine is a biological fluid containing many cytokines that can be considered predictors of poor prognosis for the progression of the renal damage.[46, 47] Today, proteomics-based techniques, are promising approaches for uncovering new and more sensitive and relevant biomarkers that may be involved in the earlier phases of IgAN. Several studies have identified specific polypeptide patterns in patients' urine, as reported in supplementary table 9 . Other proteins are able to differentiate IgAN from other diseases or distinguish specific proteomic profiles on the basis of disease severity or identify specific alterations during the progression of renal damage. Other urinary proteomics patterns distinguish progressor from non-progressor IgAN patients. Some urinary proteins are also predictive of inadequate response to ACEi.

Data from the published articles do not evidence a common urinary pattern of polypeptides in the urine of IgAN patients probably due to different cohorts of patients included in the studies. However, some common proteins such as fragments of albumin, alfa1 antitrypsin, uromodulin, alfa1-microglobulin have been reported by many investigators.

Limitations of the published studies are (i) different cohorts of IgAN patients included in the studies, (ii) different grades of renal damage; (iii) different techniques used for detecting urinary polypeptides; (iv) absence of validation in independent IgAN cohorts. Future studies should propend in the identification of a proteome based classifier containing specific peptides able to discriminate IgAN patients with specific clinical patterns (i.e. microscopic hematuria or patients with recurrent episodes of macroscopic hematuria) from healthy individuals and from other types of glomerulonephritis. The same classifier could be used as a disease outcome indicator or could be helpful in the evaluation of a therapeutic response. A urinary proteome based classifier CKD273 has successfully been identified in diabetic nephropathy and used in a

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prospective randomised clinical trial.[48] This proteomic panel of 273 urinary peptides performed significantly better than albuminuria in predicting the early stages of CKD,[49]; a similar approach is needed in IgAN.

A recent development in the proteomic analysis is the new high-throughput Imaging Mass Spectrometry (IMS) technique for the identification, quantization and distribution of proteins, lipids and chemical metabolites at a picomol level in complex multicellular tissue,[50] this procedure could be applied for the evaluation of biopsy sections and help differentiate IgAN sub-phenotypes. Furthermore, IMS has the power to discover specific 3D peptide profiles that are constitutively expressed in human cells and tissue types and in the future the IMS integration with other OMICs such as genomics and transcriptomics will generate specific gene-transcript-protein networks in health and disease. [51, 52]

1.5 METABOLOMICS

Metabolomics studies the individual metabolic profiles and their changes overtime due to physiological and pathological conditions (Supplementary Table 10). ¹HNMR spectroscopy and pattern recognition analysis have been used by Del Coco et al to detect minor alterations in the metabolomic profile of urine from IgAN patients.[53] Data from NMR urine analysis evidenced altered values of specific metabolites (increased values of creatinine, TMAO, betaine and acetate, and decreased levels of hippurate, lactate and citrate). These results were confirmed in another study using the metabolomic urinary profiles of four primary glomerulonephrites where a specific urinary signature for IgAN patients was found.[54] Recently, Kalantari et al identified several urinary metabolite biomarkers correlating with proteinuria and the most significant pathway associated with disease severity was the phenylalanine metabolic pathway.[55]

These limited number of studies demonstrate a specific metabolite signature in IgAN correlating with the severity of the renal damage. The presence of these metabolites in the urine reflects an altered metabolic pathway determined by an altered cell activity highlighting new targets for specific therapy. However to confirm the validity of these results , data should be validated in larger independent and well phenotyped cohorts. Moreover, the integrated analysis of genomics and urinary metabolomics could be considered a promising approach for uncovering novel gene to disease connections via metabolic traits highlighting novel hypotheses about molecular mechanisms involved in the etiology of disease.[56] [57]

1.6 CONCLUDING REMARKS

A summary of the results obtained from the omic studies including points of weaknesses and strengths are described in Table 1.

Large-scale GWASs have been successfully applied in IgAN with extensively replicated results. To date GWASs have identified highly relevant associated loci containing MHC regions, the complement system and genes involved in mucosal IgA production, innate immunity and inflammatory response.[11] Fine-mapping studies are needed to uncover the causal genetic variants underlying the signals. Furthermore, identified rare/common variants underlying the IgAN phenotype must be validated with classical biological experimental approaches.

The clinical approaches using FLOW-cytometry, Western blot, ELISA, Immunohistochemistry, transfection experiments, and others have been applied in transcriptomics/epigenomics/proteomics studies, but results require extensive replication. Moreover, metabolomics results must be replicated and applied on larger cohorts of well phenotyped IgAN patients.

Results from different omics seem to be fragmented, but overlaps are evident when we focalize on the four hit pathogenic model (Table 2). High circulating levels of Gd-IgA1 (*hit 1*) are determined by a dysregulation in antigen handling and aberrancies in IgA1 production at mucosal sites.[58] All omics performed on PBLs show the involvement of altered innate and adaptive immunity. The altered response to an antigenic challenge clearly emerges during the gross hematuria episode[28] with the up-regulation of interferon-regulated genes involved in the degradation and processing of antigens (*TAP1*, *TAP2*, *PSMB8*, *PSMB9*).[28] These genes are found in highly replicated GWAS loci[11] and have been validated with traditional experimental approaches by different authors.[27, 59] Another pathway that seems to be hyper-activated in this context is WNT- β -catenin, PI3K/Akt pathway mainly determined by the downregulation of the negative regulator *PTEN* and enhanced translocation of the effector molecule NF- κ B.[27, 59] GWAS, rare genetic variants and epigenetic studies support this hyperactivation with NF- κ B translocation occurring through *VAV3*,[60] *CARD9*[61] and *miR-374b*,[41] the latter modulating a B cell proliferation and aberrant IgA1 glycosylation. Interestingly, other miRNAs let-7b and miR-148b have also been demonstrated to regulate these pathways, let-7b directly regulates *PTEN* and miR-148b regulated both *INVS* and *PTEN*. [39] The same let-7b, miR-148b, *miR-374b* participate directly in the aberrant glycosylation process targeting respectively *GALNT2*, *C1GALT1*, *C1GALT1C1-COSMC*, important enzymes in the IgA1 glycosylation process.

The Gd-IgA1 exposes N-acetylgalactosamine within IgA1 hinge-region and is an epitope for the anti-glycan response[62] that promotes the formation of circulating immune complexes (*hit 2*). This autoimmune phenotype could be regulated by MHC class II loci identified by GWAS (*HLA-DQA1*, *HLA-DQB1*, *HLA-DRB1*, *HLA-DP*) and triggered by the exposure to infectious or dietary antigens that have been aberrantly

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processed. Furthermore, also the immunoproteasome (PSMB8, PSMB9, PSMB10, TAPBP) could be involved in this process as it is highly up-regulated in a number of diseases including autoimmune diseases.- [63]

The formation of circulating immune complexes and deposition (*hit 3*) may be elicited by the hyperactivation of PI3K/AKT pathway. This pathway activates FcαRI (CD89) in monocytes.[64, 65] Then, circulating immune complexes aggregate this receptor inducing the shedding of the extracellular domain to form circulating IgA1–FcαRI complexes. Other receptors that are known to be involved in the deposition process (such as transglutaminase-2 , CD89, transferrin receptor, plgR) have not been confirmed by omic studies.

Immuno-complex deposition induces mesangial cell proliferation, secretion of extracellular matrix chemokines and cytokines with an activation of the alternative complement pathway (*hit 4*). These pathways all seem to be evident in GWAS loci and gene expression studies on kidney tissue. In particular, the activation of ITGAX-ITGAM, members of the complement system, encode for integrins αM and αX that combine with integrin β2 chain to form leukocyte-specific complement receptors 3 and 4, these receptors may augment glomerular inflammation. Toll-like receptor signalling, is also common to different omics and may be involved in the progression of the disease.

1.7 FUTURE DIRECTIONS

The use of molecular profiling technologies has identified different molecular signatures in IgAN associated with different phases of the disease and the progression of histologic lesions. The integration of data using an extensive computational support and statistical modelling is the key for connecting -omic data-sets with the clinical data[66] and for decoding the pathophysiological disease processes; ~~it is~~ ~~which is~~ necessary for an accurate and precise diagnosis and a more targeted therapy (Figure_1). Future omics studies should be applied on well phenotyped IgAN patients with extensive clinical data and MEST-C classification of renal damage. Common and rare gene variants located in the GWAS loci, PBMC gene expression data, molecular signature of renal lesions, clinical data (serum levels of aberrantly glycosylated IgA1, miRNAs, eGFR and proteinuria, morphologic pictures) urinary polypeptides and metabolites should all be integrated for the identification of diagnostic biomarkers in IgAN and risk prediction score. The integrative analyses may be facilitated by the use of online resources like Nephroseq (<https://www.nephroseq.org/resource/login.html>) and KUPKB(kidney and urinary pathway knowledge base <http://www.kupkb.org>) and other tools based on the ‘in silico’ nanodissection (<http://nano.princeton.edu>).

To date high-throughput omics analyses are in progress; different issues have to be taken into consideration to discuss a concrete methodology for personalized health care starting from OMICs

data.[67] From a technical point of view, sample acquisition and data analysis should also be standardized and results need to be validated on different platforms in large cohorts of patients. Furthermore, the study biological component systems via the computational and mathematical modeling of complex biological systems,[68] is the missing link between OMICS and precision medicine in IgAN. This methodology is crucial to provide insights into new pathways and networks between OMICS to drive innovation through biology-based computational analysis that can detect aberrant networks at the onset of the disease and to enable patient stratification on the basis of their individual genetic and molecular profiles.[69] The shift from the traditional 'trial-and-error' approach to precision medicine is possible if systems biology and multidisciplinary approaches are undertaken as occurred in other diseases.[70]

In conclusion, the integration of ~~data~~ results from and ~~results from~~ multi-level, high-dimensional data-sets is the next step for a better understanding of molecular networks and provides a unique resource for identifying new drug targets ~~systems pharmacology~~. Ultimately this methodology will lead to ~~the identification of new molecular targets and~~ personalized therapeutic approaches in different subsets of IgAN patients thus promoting precision nephrology.

Conflict of interest statement.

None declared.

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FIGURE

Figure 1. Integrative analysis of clinical findings and omics data for a better molecular understanding of IgA Nephropathy leading towards precision nephrology and improvement in disease management (personalized therapy). RCTs: Randomized Controlled Trials; eGFR: Estimated Glomerular Filtration rate; SNPs: Single Nucleotide Polymorphisms; miRNAs: microRNA

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Table 1. Overview of Omics studies in IgA Nephropathy

| OMICS | INPUT MATERIAL | TISSUE ORIGIN | STUDIES No. | RESULTS | WEAKNESSES | STRENGTHS | INVOLVED GENES/PATHWAYS |
|-----------------|----------------|-------------------|-------------|---------------------------------|---|---|--|
| GENOMICS | DNA | WB | 5 | 51 CVs | no validation with classical biological experimental approaches | -large sample size -13 replicated CVs in Asian and European populations | HLA-DRB1, HLA-DQA, HLA-DQB, CFHC,FHR3, CFHR1, CFHR4, CFHR2, CFHR5, TAP1,TAP2, PSMB8, PSMB9,VAV3 . HORMAD2, MTMR3, LIF, OSM,HLA-DR,TNFSF13, MPDU1, EIF4A1.CD68,TP53, SOX15, ITGAM-ITGAX , HLA-DP, HLA-A, HLA-DQA/B,DEFAACCS,KLF10/ODF1,CARD9,HLA-DQ, ST6GAL1,HLA-DPB1,HLA-DPA2 |
| | DNA | WB | 4 | CNVs | no validation with classical biological experimental approaches | -CFH and Defensin loci have been replicated - CNV real-time PCR assay validation for GALNT13, COL11A2, TLR9 and validation with classical biological experimental approaches and replication in Greek population | CFHR3, CFHR1, <i>HLA-DQB1</i> , <i>GALNT13</i> , <i>COL11A2</i> , TLR9 , <i>DEFA3</i> |
| | DNA | WB | 3 | 31 RVs | RVs causality validation is missing | RVs within previously validated aberrant networks | MAPK/ERK pathway, PI3K/AKT pathway |
| TRANSCRIPTOMICS | RNA | PBL | 3 | 470 Dysregulated Genes | -Results have not been replicated -small sample size | validated with classical biological experimental approaches | WNT- β -catenin and PI3K/Akt pathway (INVS , PTEN) , Enhanced proliferation of PBMCs . Innate immunity activation PSMB8, PSMB9 and TAPBP |
| | RNA | Blood CD14+ cells | 1 | 710 differently expressed genes | -Results have not been replicated -small sample size | validated with classical biological experimental approaches | TNF, CD83, NDUFS3 and TNFRSF1A |
| | RNA | KIDNEY | 4 | 140 differently expressed genes | -Results have not been replicated -small sample size | Some validated with classical biological experimental approaches | innate immune response, classical complement pathway activation and matrix turnover. Role of pattern recognition receptors in recognition of Bacteria and Viruses, Leukocyte extravasation signalling, TREM Signaling-ITGAX, NF-kB activation by viruses, Toll-like receptor Signaling , Cell cycle: G2/M DNA damage checkpoint regulation, IL-8 signaling, Production of nitric oxide and reactive oxygen species in macrophages |

| | | | | | | | |
|--------------|---------------------------|-------------------|----|---|--|--|--|
| EPIGENOMICS | DNA | Blood CD4+CELLS | 1 | Aberrantly methylated genes: DUSP3, TRIM27, VTRNA2-1, | -Results have not been replicated -small sample size | validated with classical biological experimental approaches | T helper cell imbalance towards the Th1 subtype |
| | DNA | PBMCs | 1 | Aberrantly methylated genes: COSMC | -Results have not been replicated -small sample size -results obtained from an heterogeneous cell population | validated with classical biological experimental approaches | COSMC methylation involved in aberrant glycosylation of IgA1 |
| | Total RNA including miRNA | PBMCs/SERUM | 2 | 37 dysregulated miRNAs | -small sample size | validated with classical biological experimental approaches replicated CVs in Asian and European populations | -miRNAs involved in aberrant glycosylation of IgA1 -let-7d directly regulates PTEN and miR-148b regulated both INVS and PTEN , |
| | Total RNA including miRNA | CIRCULATING CELLS | 1 | 1 upregulated miRNA | -small sample size | validated with classical biological experimental approaches | B cell proliferation by targeting PTEN miRNAs involved in aberrant glycosylation of IgA1 targeting COSMC in B cells of IgAN. |
| | Total RNA including miRNA | KIDNEY | 6 | 14 | -small sample size -no validation with classical biological experimental approaches | | Abnormal miRNA expression patterns correlated with glomerular sclerosis and interstitial fibrosis and with progression of renal damage involving complement pathway(ITGAM-ITGAX) |
| PROTEOMICS | PROTEINS | URINE | 14 | 245 | -small sample size | 3 studies have been performed validations in independent cohorts | Altered albumin, alfa1 antitrypsin, uromodulin, alfa1-microglobulin,kininogen |
| METABOLOMICS | METABOLITES | URINE | 3 | | -small sample size - no validation with classical biological experimental approaches | | Increased values of creatinine, TMAO, betaine and acetate, and decreased levels of hippurate, lactate and citrate |

Abbreviations: **CNVs:** Copy number variations; **CVs:** common variants; **PBL:** peripheral blood leukocytes; **PBMCs:** Peripheral Blood Mononuclear Cells; **RVs:** rare variants; **WB:** whole blood;

*In **bold** genes or pathways observed in more than one study.

Table 2 Omics studies in the context of the four hit pathogenic model

| HIT | PHASE PROCESS | MOLECULAR PROCESSES INVOLVED | OMICS CONTRIBUTIONS | RELATED OMICS PATHOLOGICAL PROCESSES |
|-----|---|--|----------------------------|--|
| 1 | High circulating levels of galactose-deficient IgA1 (Gd-IgA1) | Dysregulation of IgA1 production at mucosal surfaces alterations in posttranslational modification of O-glycans within IgA1-producing cells. | common genetic variants | <i>HORMAD2</i> , <i>LIF</i> , <i>OSM</i> , <i>DEFA</i> , <i>TNFSF13</i> genes appear to modulate mucosal immunity and production of IgA1. <i>VAV3</i> essential for adaptive immune function and NF- κ B activation in B-cells, a process that stimulates IgA production. <i>ITGAM</i> and <i>ITGAX</i> are involved in intestinal inflammation and IgA production. <i>CARD9</i> activates NF- κ B, which is responsible for both innate and adaptive immunity. <i>TAP1</i> , <i>TAP2</i> , <i>PSMB8</i> , <i>PSMB9</i> are involved in the antigen presentation pathway. MHC class II alleles loci (<i>HLA-DQA1</i> , <i>HLA-DQB1</i> , <i>HLA-DRB1</i> , <i>HLA-DP</i>) may participate in the regulation of intestinal inflammation and IgA production. |
| | | | rare genetic variants | - Multiple rare genetic variants co-segregating with familial IgA nephropathy all act within a single immune-related network where WNT- β catenin, PI3K/Akt pathway and interferon signalling involve both innate and adaptive immunity |
| | | | gene expression studies | -Hyperactivation of WNT- β catenin and PI3K/Akt pathways (down regulation of <i>INVS</i> and <i>PTEN</i>) leads to a defect in antigen handling and to abnormal systemic responses to mucosally encountered antigens. -Defect in antigen handling in PBMCs has been demonstrated during the macroscopic haematuria with a specific up-regulation of the immunoproteasome pathway (<i>PSMB8</i> , <i>PSMB9</i> , <i>PSMB10</i> , <i>TAPBP</i>) |
| | | | miRNA & metilation studies | -miRNA let-7b and miR-148b modulate two enzymes involved in the sequential O-glycosylation process of the IgA1 molecule. Abnormal miRNA-based regulatory mechanism influence the O-glycosylation process determining the aberrant glycosylation of IgA1. Furthermore, let-7d directly regulates <i>PTEN</i> and miR-148b regulates both <i>INVS</i> and <i>PTEN</i> ,- |
| | | | | -Upregulation of miR-374b promotes B cell proliferation and aberrant IgA1 glycosylation by targeting <i>Cosmc</i> and <i>PTEN</i> -The gene expression of <i>Cosmc</i> , whose activity is closely related to Gd-IgA1, is regulated by DNA methylation in lymphocytes - Aberrantly methylated regions in CD4+ T-cells of IgAN patients led to the altered expression of genes involved in <i>TCR</i> signal transduction and in the reduced TCR signal strength explaining the T-helper cell imbalance towards the Th1 subtype. |

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|---|---|--|----------------------------|--|
| 2 | Production of anti-Gd-IgA1 autoantibodies of IgG and/or IgA isotype | High Gd-IgA1 elicits an autoimmune response, resulting in generation of anti-glycan antibodies that recognize N-acetylgalactosamine epitopes on Gd-IgA1 | common genetic variants | Anti-glycan response may be triggered by the exposure to infectious or dietary antigens that have been aberrantly processed by genetically predisposing MHC class II allele loci: <i>HLA-DQA1</i> , <i>HLA-DQB1</i> , <i>HLA-DRB1</i> , <i>HLA-DP</i> having a role in autoimmunity |
| | | | gene expression studies | Defect in antigen handling in PBMCs with an up-regulation of interferon signalling and immunoproteasome pathway (PSMB8, PSMB9, PSMB10, TAPBP) has been demonstrated in IgAN patients. These pathways seem to be upregulated in autoimmune diseases |
| 3 | Formation of circulating IgA1–IgG and IgA1–IgA1 immune complexes and deposition | Immune complexes activate alternative complement pathway | common genetic variants | ITGAM is essential for interactions between CD89 and secretory IgA |
| | | | gene expression studies | -Hyperactivation of PI3K pathway in monocytes activates FcαRI and circulating immune complexes aggregate this receptor. This interaction induces shedding of the extracellular domain to form circulating IgA1–FcαRI complexes that could have a pathogenic role in IgAN. - Gene expression studies on kidney tissue have evidenced ITGAM |
| 4 | Local activation of inflammatory pathways and the complement system | local inflammation, mesangial cell proliferation, secretion pro-inflammatory cytokines leading to the interstitial infiltration of inflammatory cells and promoting glomerular and tubulointerstitial fibrosis | common genetic variants | - Glomerular inflammation is enhanced by the complement system - lectin or the alternative pathway (CFHR3,1-del, CFHR1 and CFHR3) - ITGAM and ITGAX encode integrins that combine to form leukocyte specific complement receptor CR3 and CR4. These may be involved in glomerular inflammation. |
| | | | gene expression studies | -Various gene expression studies conducted on kidney tissue show the involvement of relevant signalling pathways: Activation Complement system (<i>ITGAX-ITGAM</i> , <i>C1QA</i> , <i>C1QB</i>); Toll-like receptor Signalling; NF-kB pathway; IL-8 signalling; Production of nitric oxide and reactive oxygen species in macrophages; -Kidney production of cytokines (mainly IL6) promotes mesangial cell proliferation |
| | | | miRNA & metilation studies | -TLR9 CNV contributes to the deterioration of the renal function |
| | | | Proteomics | -Low levels of UMOD peptides cause progression of renal damage |

Omics studies for Comprehensive Understanding of IgA Nephropathy. State-of-the-Art and Future Directions

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Summary

IgA Nephropathy (IgAN) is the most common worldwide primary glomerulonephritis with a strong autoimmune component . The disease shows variability in both clinical phenotypes and endpoints, and can be potentially subdivided into more homogenous subtypes through the identification of specific molecular biomarkers. This review focuses on the role of -omics in driving the identification of potential molecular subtypes of the disease through the integration of multi-level data from genomics, transcriptomics, epigenomics, proteomics and metabolomics. First, the identification of molecular biomarkers, including mapping of the full spectrum of common and rare IgAN risk alleles, could permit a more precise stratification of IgAN patients. Second, the analysis of transcriptomic patterns and their modulation by epigenetic factors like miRNAs have the potential to increase our understanding in the pathogenic mechanisms of the disease. Third, the specificity of urinary proteomic and metabolomic signatures and the understanding of their functional relevance may contribute to the development of new non-invasive biomarkers for a better molecular characterization of the renal damage and its follow-up. All these approaches can give information for targeted therapeutic decisions and will support novel clinical decision making. In conclusion, we offer a framework of omic studies and outline barriers and potential solutions that should be used for improving the diagnosis and treatment of the disease. The ongoing decade is exploiting novel high-throughput molecular technologies and computational analyses for improving the diagnosis (precision nephrology) and treatment (personalised therapy) of the IgAN subtypes.

Key words

IgA nephropathy, genomics, epigenomics, transcriptomics, proteomics, metabolomics,

Running title :Omics in IgA Nephropathy

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Introduction

IgA Nephropathy (IgAN) is the most common primary glomerulonephritis worldwide [1, 2] that develops mainly in the 2nd and 3rd decade of life and 40% of IgAN cases reach end-stage kidney disease (ESKD) after 20 years from the biopsy-proven diagnosis implying a great socio-economical burden.[3, 4] In this review, we describe the results of omics studies carried out in IgAN patients. We move from genomic studies focusing on common and rare variants linked to the disease, to transcriptomics carried out on circulating immune cells and kidney biopsy specimens and then to epigenomics, proteomics and metabolomics . Finally, we suggest an integrative approach for developing biological networks and identify potential diagnostic biomarkers for Precision Nephrology and Personalized Therapy.

1.1 GENOMICS

Two main genome wide genetic approaches have been used in the study of this complex disease: Genome Wide Association Studies (GWAS) and Next Generation Sequencing (NGS).

1.1.1 Common Variants

The GWAS conducted on IgAN have identified several common variants that clearly show a strong participation of the *Human Leukocyte Antigen* (HLA) system, genes involved in innate immunity, and other loci summarized in Supplementary Table 1. Seven Single-Nucleotide Polymorphisms (SNPs) identified through GWAS were used for constructing a genetic risk score, which explained 4.7% of overall IgAN risk.[5] This score was integrated with other 8 recently associated SNPs, but it cumulatively explains only 5.37% of disease risk (http://www.columbiamedicine.org/divisions/gharavi/calc_genetic.php). [6] In summary, IgAN risk loci identified through GWAS i) are common to other inflammatory and immune-mediated diseases, ii) explain a proportion of the disease risk worldwide, iii) contribute to the geographic variation in disease prevalence, iv) confirm the polygenic and multiple-susceptibility-gene nature of IgAN. Future studies are needed to evaluate if these GWAS signals are effectively generated by causative common variants or due to rare variants in linkage disequilibrium with the common ones. Direct sequencing of the associated regions may lead to a finer mapping of these disease-related loci, as seen for example in chronic obstructive pulmonary disease. [7]

Very recently two quantitative GWAS have identified two loci, in *core 1*, *β1-3-galactosyltransferase-1* (*C1GALT1*) and *C1GALT1 Specific Chaperone 1* (*C1GALT1C1-cosmc*)[8, 9] providing novel insights into the

genetic regulation of O-glycosylation and providing evidence that also common genetic variations can influence O-glycosylation.

Copy Number Variants (CNVs) in IgAN have been reported in complement Factor H Related proteins(*CFHR1*, *CFHR3*) [10, 11] and have been associated with decreased IgAN risk (supplementary Table 2). CNVs were also found enriched in the linked 17q12-22 locus [12, 13] and in the *Toll Like Receptor 9 (TLR9) gene* where its potential role in disease progression was confirmed.[13] A lower copy number of three variants within the defensin highly variable locus correlated with renal dysfunction, increased serum IgA1 and Gd-IgA1.[14, 15]

1.1.2 Rare variants

Rare variants, usually found in the frequency range between 0.1% and 1%, are more likely to have a stronger effect in complex diseases compared to common variants. Three Whole Exome Sequencing (WES) studies have been performed on IgAN (Supplementary Table 3). The first study evidenced seven common co-segregating deleterious variants within four genes (*CARD8*, *DEFA4*; *MYCT1*, *ZNF543*).[16] The second WES study performed on two affected individuals and an unaffected familial control from a large Sicilian family with multiple affected individuals, identified a novel missense variant in the *Sprouty RTK Signaling Antagonist 2 (SPRY2)* gene that segregated in all IgAN affected individuals.[17] Functional analysis of the variant in B lymphoblastoid cell lines from affected members linked the *SPRY2* mutation showed the inhibition of the MAPK/ERK pathway. Recently, a combined linkage analysis and exome sequencing methodology has been successfully applied for pinpointing specific causative variants involved in familial goiter.[18] A similar procedure has been carried out by Cox et al. In the last IgAN study and their results support a polygenic and a multiple-susceptibility-gene model for familial IgAN.[19] Evident connections with previous gene expression studies were found, but further studies are needed to understand whether these variants can effectively disrupt gene function and validate their causality in contributing to IgAN phenotype. Furthermore, these studies must be replicated in other independent cohorts of IgAN patients to confirm the validity of the results.

To date several efforts have been made to identify susceptibility variants, but NGS data for the identification of rare variants within IgAN GWAS areas has never been done. This strategy has been recently applied in the study of age-related macular degeneration in which rare causative coding variants were pinpointed within known associated genetic loci and could represent an innovative and promising approach for IgAN.[20] Association testing deployed in parallel or in combination to familial linkage could represent another innovative strategy for the identification and characterization of a full range of disease-susceptibility variants as seen in hearing impairment and cardiomyopathy .[21-24]

1.2 TRANSCRIPTOMICS

Transcriptome is considered the gene signature leading to a phenotype that is possibly influenced by genetic determinants. Specific gene expression patterns have been found in IgAN patients' blood cells. (Supplementary Table 4). An aberrant modulation of genes belonging to the WNT- β -catenin and PI3K/AKT pathways was found in peripheral blood leukocytes (PBL) IgAN patients.[25] Monocytes were principally involved in the altered WNT signaling pathway and an expansion of the non-classical CD16⁺ monocyte subset characterized by an enhanced apoptotic potential was demonstrated.[26] Moreover, transcriptomics was used to analyze gross hematuria episodes in concomitance to mucosal infections, an important time point for IgAN.[27, 28] Differently regulated genes during the gross hematuria episode were principally involved in interferon signaling and antigen presentation.[28] This network showed an up-regulation of genes involved in the immune-proteasome pathway and based on a series of additional experimental approaches, *C-X3-C Motif Chemokine Receptor 1 (CX3CR1)* and its ligand fractalkine were found to promote macroscopic hematuria in IgAN patients. An important limitation of all these experimental approaches is that gene expression data have not been integrated with genome wide genotype data. This integration could be useful for the identification of expression quantitative trait loci (eQTL) giving a direct genetic explanation of aberrant gene expression data, useful for translational medicine.[29]

Most transcriptomic findings have been obtained from complex starting material i.e whole blood, which is made up of different cell lineages. Future studies should propend towards the use of cell sorting technologies for analyzing the transcriptomic profile of specific cell populations or single cell analysis may give novel insights into health and disease status.[30] These novel technological approaches may help to obtain cell-lineage specific expression data from IgA1 secreting cells, giving important insights into the pathogenic mechanisms involved in the disease.

The renal damage in IgAN is caused by mesangial deposition of polymeric IgA1 and or IgA1-IgG immune complexes at glomerular level leading to oversynthesis of extracellular matrix (ECM). Since alterations in mRNA levels could precede the histological damage, transcriptomics in kidney tissue might identify gene expression profiles involved in the development and progression of renal damage.

Investigators focused their attention on the gene expression of isolated glomeruli or tubulointerstitial tissue from IgAN patients' kidney biopsies. They observed an increased expression of some proteoglycans directly involved in renal damage and suggested their potential role as prognostic biomarkers and highlighted an 11 transcript proteinuria signature in the tubulo-interstitial compartement.[31, 32]

Differently expressed genes in microdissected glomeruli with endocapillary proliferation were involved in innate immune response, classical complement pathway activation and matrix turnover. Interestingly, the in-silico study demonstrated that the aberrantly expressed genes characterizing endocapillary proliferation were responsive to the combined corticosteroid-resveratrol therapy.[33] A subsequent study demonstrated that altered expressed genes were responsive to some organic substances such as doxorubicin and thapsigargin[34] (see supplementary Table 5).

Data from these studies indicate that (i) glomerular and tubulointerstitial gene expressions are independent; (ii) some genes involved in specific pathways are responsive to drugs. Gene expression data from isolated glomeruli or tubules are not correlated to the extent of renal damage but it's a sterile description of up and down-regulated genes. Future studies should be performed on the entire tissue as whole since kidney disease involves all renal compartments globally and simultaneously. In particular, gene expression studies will need to be performed on well characterized kidney biopsy specimens that have been accurately scored with the new reproducible MEST-C classification system.[35] In this way specific gene expression changes that characterize active renal lesions (E and C) that are more responsive to immunosuppressive therapy compared to chronic lesions (S and T) will be identified. Furthermore, differences in gene expression linked to the degree of histologic renal damage have yet to be evaluated. This information is important because glomerular lesions are always associated with tubulointerstitial damage during the progression of renal damage.

The emerging transcriptomic methodology not deployed in the study of IgAN is RNAseq. This methodology has brought a significant qualitative and quantitative improvement to transcriptome analysis, offering an unprecedented level of resolution able to detect genes expressed at low levels, splice isoforms and novel exons/genes.[36] In addition, allele-specific expression, RNA editing and fusion transcripts represent some of the information that do not emerge from hybridization-based platforms and may be crucial in complex diseases.[37]

1.3 EPIGENOMICS

To date, studies on DNA methylation in IgAN are principally two (Supplementary Table 6). The first study showed that the gene expression of *Cosmc*, whose activity is closely related to aberrantly glycosylated IgA1, could be regulated by DNA methylation in lymphocytes of children with IgAN.[38] Limitations of this study, in addition to the small sample size, are the lack of strong validation, functional assays and DNA methylation analysis using a mixed cell population peripheral blood mononuclear cells (PBMCs). The second study showed that the two hypomethylated DNA regions contained the promoters for *Dual Specificity*

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3 *Phosphatase 3 (DUSP3)* and *Tripartite Motif Containing 27 (TRIM27)* and the most extensively
4 hypermethylated region on chromosome 5 contained the *Vault RNA 2-1 (VTRNA2-1)* gene promoter, known
5 as the precursor of miR-886. These aberrantly methylated DNA regions were found to induce a T helper cell
6 imbalance towards the Th1 subtype. Even if the sample size is not very large, this study can be considered
7 confident due to the many validation experiments and functional assays.
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11 MicroRNAs (miRNAs) are another epigenetic component that can modulate gene expression in tissue and
12 biological fluids. Three different genome-wide miRNA gene expression studies on IgAN patients' blood cells
13 have been performed (see Supplementary table 7). The first study led to the identification of two
14 aberrantly expressed miRNAs (let-7b and miR-148b) that regulate the gene expression of two key enzymes
15 involved in the sequential O-glycosylation process of the IgA1 molecule (*GALNT2*, *C1GALT1*). [39] These
16 results were validated biologically with transient transfection experiments and demonstrated that the
17 abnormal miRNA-based regulatory mechanism influences the O-glycosylation process determining the
18 aberrant glycosylation of IgA1, main characteristic of IgAN. [39, 40] These findings could be exploited for
19 therapeutic use because the inhibition of these upregulated miRNAs could normalize the IgA1 O-
20 glycosylation process.
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24 Recently, the miRNA expression profiling of B cells from IgAN patients demonstrated an upregulation of
25 miR-374b that is able to determine B cell proliferation and aberrant IgA1 glycosylation by targeting
26 *C1GALT1C1-Cosmc* and *Phosphatase and Tensin Homolog (PTEN)*; [41] the latter has already been found
27 aberrantly modulated in IgAN. [25] Anti-miR blockers could be exploited for new therapeutic approaches
28 and are being tested in ex-vivo clinical studies on myocardial infarction, neoplasms and in phase III clinical
29 trials for hepatitis C virus infection. [41-44]
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33 While the majority of miRNAs are found within the cell, a handful of circulating miRNAs, released from
34 blood cells, have also been detected in various body fluids. In a multicenter international retrospective
35 study, let-7b and miR-148b levels were measured in serum samples of Caucasian and Asian IgAN patient
36 sand their combined value was found as a significant predictor of the disease status, showing a good
37 sensitivity and specificity and discriminating IgAN patients from other primary glomerulonephritides. [45]
38 This combined biomarker seems to be a novel, specific and non-invasive indicator to test the probability of
39 being affected by IgAN, mainly in patients' relatives with persistent urinary abnormalities.
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43 In the last years, several groups studied the role of miRNAs in kidney tissue. A cluster of miRNAs has been
44 observed in the human kidney tissue with a different distribution in renal cortex and medulla of rat kidney.
45 To date, few studies have been carried out on IgAN renal biopsies (see Supplementary table 8). Abnormal
46 miRNA expression patterns in renal biopsies correlated with glomerular sclerosis and interstitial fibrosis.
47 Various miRNAs have been found to be involved in the progression of renal damage or in the pro-fibrotic
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processes in IgAN through E-cadherin and the epithelial to mesenchymal transition process. Recently, miRNAs have also been found to have a role in the activation of mesangial cells by secretory IgA (SIgA) from IgAN patients.[43] In conclusion, regarding the use of miRNAs as biomarkers in the blood and kidney, many studies have been conducted in retrospective cohorts but prospective studies are required to confirm their clinical utility and diagnostic value in asymptomatic individuals. In addition, several miRNAs have been identified as targets for the treatment but the use of miRNA modulators in pre-clinical settings is still missing.

1.4 PROTEOMICS

Urine is a biological fluid containing many cytokines that can be considered predictors of poor prognosis for the progression of the renal damage.[46, 47] Today, proteomics-based techniques, are promising approaches for uncovering new and more sensitive and relevant biomarkers that may be involved in the earlier phases of IgAN. Several studies have identified specific polypeptide patterns in patients’ urine, as reported in supplementary table 9 . Other proteins are able to differentiate IgAN from other diseases or distinguish specific proteomic profiles on the basis of disease severity or identify specific alterations during the progression of renal damage. Other urinary proteomics patterns distinguish progressor from non-progressor IgAN patients. Some urinary proteins are also predictive of inadequate response to ACEi.

Data from the published articles do not evidence a common urinary pattern of polypeptides in the urine of IgAN patients probably due to different cohorts of patients included in the studies. However, some common proteins such as fragments of albumin, alfa1 antitrypsin, uromodulin, alfa1-microglobulin have been reported by many investigators.

Limitations of the published studies are (i) different cohorts of IgAN patients included in the studies, (ii) different grades of renal damage; (iii) different techniques used for detecting urinary polypeptides; (iv) absence of validation in independent IgAN cohorts. Future studies should propend in the identification of a proteome based classifier containing specific peptides able to discriminate IgAN patients with specific clinical patterns (i.e. microscopic hematuria or patients with recurrent episodes of macroscopic hematuria) from healthy individuals and from other types of glomerulonephritis. The same classifier could be used as a disease outcome indicator or could be helpful in the evaluation of a therapeutic response. A urinary proteome based classifier CKD273 has successfully been identified in diabetic nephropathy and used in a prospective randomised clinical trial.[48] This proteomic panel of 273 urinary peptides performed significantly better than albuminuria in predicting the early stages of CKD,[49] a similar approach is needed in IgAN.

A recent development in the proteomic analysis is the new high-throughput Imaging Mass Spectrometry (IMS) technique for the identification, quantization and distribution of proteins, lipids and chemical metabolites at a picomol level in complex multicellular tissue,[50] this procedure could be applied for the evaluation of biopsy sections and help differentiate IgAN sub-phenotypes. Furthermore, IMS has the power to discover specific 3D peptide profiles that are constitutively expressed in human cells and tissue types and in the future the IMS integration with other OMICs such as genomics and transcriptomics will generate specific gene-transcript-protein networks in health and disease. [51, 52]

1.5 METABOLOMICS

Metabolomics studies the individual metabolic profiles and their changes overtime due to physiological and pathological conditions (Supplementary Table 10). ¹HNMR spectroscopy and pattern recognition analysis have been used by Del Coco et al to detect minor alterations in the metabolomic profile of urine from IgAN patients.[53] Data from NMR urine analysis evidenced altered values of specific metabolites (increased values of creatinine, TMAO, betaine and acetate, and decreased levels of hippurate, lactate and citrate). These results were confirmed in another study using the metabolomic urinary profiles of four primary glomerulonephrites where a specific urinary signature for IgAN patients was found.[54] Recently, Kalantari et al identified several urinary metabolite biomarkers correlating with proteinuria and the most significant pathway associated with disease severity was the phenylalanine metabolic pathway.[55]

These limited number of studies demonstrate a specific metabolite signature in IgAN correlating with the severity of the renal damage. The presence of these metabolites in the urine reflects an altered metabolic pathway determined by an altered cell activity highlighting new targets for specific therapy. However to confirm the validity of these results, data should be validated in larger independent and well phenotyped cohorts. Moreover, the integrated analysis of genomics and urinary metabolomics could be considered a promising approach for uncovering novel gene to disease connections via metabolic traits highlighting novel hypotheses about molecular mechanisms involved in the etiology of disease.[56] [57]

1.6 CONCLUDING REMARKS

A summary of the results obtained from the omic studies including points of weaknesses and strengths are described in Table 1.

Large-scale GWASs have been successfully applied in IgAN with extensively replicated results. To date GWASs have identified highly relevant associated loci containing MHC regions, the complement system and genes involved in mucosal IgA production, innate immunity and inflammatory response.[11] Fine-

mapping studies are needed to uncover the causal genetic variants underlying the signals. Furthermore, identified rare/common variants underlying the IgAN phenotype must be validated with classical biological experimental approaches.

The clinical approaches using FLOW-cytometry, Western blot, ELISA, Immunohistochemistry, transfection experiments, and others have been applied in transcriptomics/epigenomics/proteomics studies, but results require extensive replication. Moreover, metabolomics results must be replicated and applied on larger cohorts of well phenotyped IgAN patients.

Results from different omics seem to be fragmented, but overlaps are evident when we focalize on the four hit pathogenic model (Table 2). High circulating levels of Gd-IgA1 (*hit 1*) are determined by a dysregulation in antigen handling and aberrancies in IgA1 production at mucosal sites.[58] All omics performed on PBLs show the involvement of altered innate and adaptive immunity. The altered response to an antigenic challenge clearly emerges during the gross hematuria episode with the up-regulation of interferon-regulated genes involved in the degradation and processing of antigens (*TAP1*, *TAP2*, *PSMB8*, *PSMB9*).[28] These genes are found in highly replicated GWAS loci[11] and have been validated with traditional experimental approaches by different authors.[27, 59] Another pathway that seems to be hyper-activated in this context is WNT- β -catenin, PI3K/Akt pathway mainly determined by the downregulation of the negative regulator *PTEN* and enhanced translocation of the effector molecule NF- κ B.[27, 59] GWAS, rare genetic variants and epigenetic studies support this hyperactivation with NF- κ B translocation occurring through *VAV3*,[60] *CARD9*[61] and *miR-374b*,[41] the latter modulating a B cell proliferation and aberrant IgA1 glycosylation. Interestingly, other miRNAs let-7b and miR-148b have also been demonstrated to regulate these pathways, let-7b directly regulates *PTEN* and miR-148b regulated both *INVS* and *PTEN*. [39] The same let-7b, miR-148b, *miR-374b* participate directly in the aberrant glycosylation process targeting respectively *GALNT2*, *C1GALT1*, *C1GALT1C1-COSMC*, important enzymes in the IgA1 glycosylation process.

The Gd-IgA1 exposes N-acetylgalactosamine within IgA1 hinge-region and is an epitope for the anti-glycan response[62] that promotes the formation of circulating immune complexes (*hit 2*). This autoimmune phenotype could be regulated by MHC class II loci identified by GWAS (*HLA-DQA1*, *HLA-DQB1*, *HLA-DRB1*, *HLA-DP*) and triggered by the exposure to infectious or dietary antigens that have been aberrantly processed. Furthermore, also the immunoproteasome (*PSMB8*, *PSMB9*, *PSMB10*, *TAPBP*) could be involved in this process as it is highly up-regulated in a number of diseases including autoimmune diseases. [63]

The formation of circulating immune complexes and deposition (*hit 3*) may be elicited by the hyperactivation of PI3K/AKT pathway. This pathway activates Fc α RI (CD89) in monocytes.[64, 65] Then, circulating immune complexes aggregate this receptor inducing the shedding of the extracellular domain to form circulating IgA1-Fc α RI complexes. Other receptors that are known to be involved in the deposition

process (such as transglutaminase-2 , CD89, transferrin receptor, plgR) have not been confirmed by omic studies.

Immuno-complex deposition induces mesangial cell proliferation, secretion of extracellular matrix chemokines and cytokines with an activation of the alternative complement pathway (*hit 4*). These pathways all seem to be evident in GWAS loci and gene expression studies on kidney tissue. In particular, the activation of ITGAX-ITGAM, members of the complement system, encode for integrins α M and α X that combine with integrin β 2 chain to form leukocyte-specific complement receptors 3 and 4, these receptors may augment glomerular inflammation. Toll-like receptor signalling, is also common to different omics and may be involved in the progression of the disease.

1.7 FUTURE DIRECTIONS

The use of molecular profiling technologies has identified different molecular signatures in IgAN associated with different phases of the disease and the progression of histologic lesions. The integration of data using an extensive computational support and statistical modelling is the key for connecting omic data-sets with the clinical data[66] and for decoding the pathophysiological disease processes; it is necessary for an accurate and precise diagnosis and a more targeted therapy (Figure 1). Future omics studies should be applied on well phenotyped IgAN patients with extensive clinical data and MEST-C classification of renal damage. Common and rare gene variants located in the GWAS loci, PBMC gene expression data, molecular signature of renal lesions, clinical data (serum levels of aberrantly glycosylated IgA1, miRNAs, eGFR and proteinuria, morphologic pictures) urinary polypeptides and metabolites should all be integrated for the identification of diagnostic biomarkers in IgAN and risk prediction score. The integrative analyses may be facilitated by the use of online resources like Nephroseq (<https://www.nephroseq.org/resource/login.html>) and KUPKB(kidney and urinary pathway knowledge base <http://www.kupkb.org>) and other tools based on the 'in silico' nanodissection (<http://nano.princeton.edu>). To date high-throughput omics analyses are in progress; different issues have to be taken into consideration to discuss a concrete methodology for personalized health care starting from OMICS data.[67] From a technical point of view, sample acquisition and data analysis should also be standardized and results need to be validated on different platforms in large cohorts of patients. Furthermore, the study biological component systems via the computational and mathematical modeling of complex biological systems,[68] is the missing link between OMICS and precision medicine in IgAN. This methodology is crucial to provide insights into new pathways and networks between OMICS to drive innovation through biology-based computational analysis that can detect aberrant networks at the onset of the disease and to enable patient stratification on the basis of their individual genetic and molecular profiles.[69] The shift from the traditional 'trial-and-error' approach

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to precision medicine is possible if systems biology and multidisciplinary approaches are undertaken as occurred in other diseases.[70]

In conclusion, the integration of results from multi-level, high-dimensional data-sets is the next step for a better understanding of molecular networks and provides a unique resource for identifying new drug targets. Ultimately this methodology will lead to personalized therapeutic approaches in different subsets of IgAN patients thus promoting precision nephrology.

Conflict of interest statement.

None declared.

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FIGURE

Figure 1. Integrative analysis of clinical findings and omics data for a better molecular understanding of IgA Nephropathy leading towards precision nephrology and improvement in disease management (personalized therapy). RCTs: Randomized Controlled Trials; eGFR: Estimated Glomerular Filtration rate; SNPs: Single Nucleotide Polymorphisms; miRNAs: microRNA

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Table 1. Overview of Omics studies in IgA Nephropathy

| OMICS | INPUT MATERIAL | TISSUE ORIGIN | STUDIES No. | RESULTS | WEAKNESSES | STRENGTHS | INVOLVED GENES/PATHWAYS |
|-----------------|----------------|-------------------|-------------|---------------------------------|---|---|--|
| GENOMICS | DNA | WB | 5 | 51 CVs | no validation with classical biological experimental approaches | -large sample size -13 replicated CVs in Asian and European populations | HLA-DRB1, HLA-DQA, HLA-DQB, CFHC,FHR3, CFHR1, CFHR4, CFHR2, CFHR5,TAP1,TAP2, PSMB8, PSMB9,VAV3. HORMAD2, MTMR3, LIF, OSM,HLA-DR,TNFSF13, MPDU1, EIF4A1.CD68,TP53, SOX15,ITGAM-ITGAX, HLA-DP, HLA-A, HLA-DQA/B,DEFAACCS,KLF10/ODF1,CARD9,HLA-DQ, ST6GAL1,HLA-DPB1,HLA-DPA2 |
| | DNA | WB | 4 | CNVs | no validation with classical biological experimental approaches | -CFH and Defensin loci have been replicated - CNV real-time PCR assay validation for GALNT13, COL11A2, TLR9 and validation with classical biological experimental approaches and replication in Greek population | CFHR3, CFHR1, HLA-DQB1, GALNT13, COL11A2, TLR9, DEFA3 |
| | DNA | WB | 3 | 31 RVs | RVs causality validation is missing | RVs within previously validated aberrant networks | MAPK/ERK pathway, PI3K/AKT pathway |
| TRANSCRIPTOMICS | RNA | PBL | 3 | 470 Dysregulated Genes | -Results have not been replicated -small sample size | validated with classical biological experimental approaches | WNT-β-catenin and PI3K/Akt pathway (INVS , PTEN), Enhanced proliferation of PBMCs. Innate immunity activation PSMB8, PSMB9 and TAPBP |
| | RNA | Blood CD14+ cells | 1 | 710 differently expressed genes | -Results have not been replicated -small sample size | validated with classical biological experimental approaches | TNF, CD83, NDUFS3 and TNFRSF1A |
| | RNA | KIDNEY | 4 | 140 differently expressed genes | -Results have not been replicated -small sample size | Some validated with classical biological experimental approaches | innate immune response, classical complement pathway activation and matrix turnover. Role of pattern recognition receptors in recognition of Bacteria and Viruses, Leukocyte extravasation signalling, TREM Signaling-ITGAX, NF-kB activation by viruses, Toll-like receptor Signaling, Cell cycle: G2/M DNA damage checkpoint regulation, IL-8 signaling, Production of nitric oxide and reactive oxygen species in macrophages |

| | | | | | | | |
|---------------------|---------------------------|-------------------|----|---|--|--|--|
| EPIGENOMICS | DNA | Blood CD4+CELLS | 1 | Aberrantly methylated genes: DUSP3, TRIM27, VTRNA2-1, | -Results have not been replicated -small sample size | validated with classical biological experimental approaches | T helper cell imbalance towards the Th1 subtype |
| | DNA | PBMCs | 1 | Aberrantly methylated genes: COSMC | -Results have not been replicated -small sample size -results obtained from an heterogeneous cell population | validated with classical biological experimental approaches | COSMC methylation involved in aberrant glycosylation of IgA1 |
| | Total RNA including miRNA | PBMCs/SERUM | 2 | 37 dysregulated miRNAs | -small sample size | validated with classical biological experimental approaches replicated CVs in Asian and European populations | -miRNAs involved in aberrant glycosylation of IgA1 -let-7d directly regulates PTEN and miR-148b regulated both INVS and PTEN , |
| | Total RNA including miRNA | CIRCULATING CELLS | 1 | 1 upregulated miRNA | -small sample size | validated with classical biological experimental approaches | B cell proliferation by targeting PTEN miRNAs involved in aberrant glycosylation of IgA1 targeting COSMC in B cells of IgAN. |
| | Total RNA including miRNA | KIDNEY | 6 | 14 | -small sample size -no validation with classical biological experimental approaches | | Abnormal miRNA expression patterns correlated with glomerular sclerosis and interstitial fibrosis and with progression of renal damage involving complement pathway(ITGAM-ITGAX) |
| PROTEOMICS | PROTEINS | URINE | 14 | 245 | -small sample size | 3 studies have been performed validations in independent cohorts | Altered albumin, alfa1 antitrypsin, uromodulin, alfa1-microglobulin,kininogen |
| METABOLOMICS | METABOLITES | URINE | 3 | | -small sample size - no validation with classical biological experimental approaches | | Increased values of creatinine, TMAO, betaine and acetate, and decreased levels of hippurate, lactate and citrate |

Abbreviations: CNVs: Copy number variations; CVs: common variants; PBL: peripheral blood leukocytes; PBMCs: Peripheral Blood Mononuclear Cells; RVs: rare variants; WB: whole blood;

*In **bold** genes or pathways observed in more than one study.

Table 2 Omics studies in the context of the four hit pathogenic model

| HIT | PHASE PROCESS | MOLECULAR PROCESSES INVOLVED | OMICS CONTRIBUTIONS | RELATED OMICS PATHOLOGICAL PROCESSES |
|-----|---|--|----------------------------|---|
| 1 | High circulating levels of galactose-deficient IgA1 (Gd-IgA1) | Dysregulation of IgA1 production at mucosal surfaces alterations in posttranslational modification of O-glycans within IgA1-producing cells. | common genetic variants | <i>HORMAD2</i> , <i>LIF</i> , <i>OSM</i> , <i>DEFA</i> , <i>TNFSF13</i> genes appear to modulate mucosal immunity and production of IgA1. <i>VAV3</i> essential for adaptive immune function and NF-κB activation in B-cells, a process that stimulates IgA production. <i>ITGAM</i> and <i>ITGAX</i> are involved in intestinal inflammation and IgA production. <i>CARD9</i> activates NF-κB, which is responsible for both innate and adaptive immunity. <i>TAP1</i> , <i>TAP2</i> , <i>PSMB8</i> , <i>PSMB9</i> are involved in the antigen presentation pathway. MHC class II alleles loci(<i>HLA-DQA1</i> , <i>HLA-DQB1</i> , <i>HLA-DRB1</i> , <i>HLA-DP</i>) may participate in the regulation of intestinal inflammation and IgA production. |
| | | | rare genetic variants | - Multiple rare genetic variants co-segregating with familial IgA nephropathy all act within a single immune-related network where WNT-βcatenin, PI3K/Akt pathway and interferon signalling involve both innate and adaptive immunity |
| | | | gene expression studies | -Hyperactivation of WNT-β-catenin and PI3K/Akt pathways (down regulation of <i>INVS</i> and <i>PTEN</i>) leads to a defect in antigen handling and to abnormal systemic responses to mucosally encountered antigens. -Defect in antigen handling in PBMCs has been demonstrated during the macroscopic haematuria with a specific up-regulation of the immunoproteasome pathway (<i>PSMB8</i> , <i>PSMB9</i> , <i>PSMB10</i> , <i>TAPBP</i>) |
| | | | miRNA & metilation studies | -miRNA let-7b and miR-148b modulate two enzymes involved in the sequential O-glycosylation process of the IgA1 molecule. Abnormal miRNA-based regulatory mechanism influence the O-glycosylation process determining the aberrant glycosylation of IgA1. Furthermore, let-7d directly regulates <i>PTEN</i> and miR-148b regulates both <i>INVS</i> and <i>PTEN</i> ,- |
| | | | | -Upregulation of miR-374b promotes B cell proliferation and aberrant IgA1 glycosylation by targeting <i>Cosmc</i> and <i>PTEN</i> -The gene expression of <i>Cosmc</i> , whose activity is closely related to Gd-IgA1, is regulated by DNA methylation in lymphocytes - Aberrantly methylated regions in CD4+ T-cells of IgAN patients led to the altered expression of genes involved in <i>TCR</i> signal transduction and in the reduced TCR signal strength explaining the T-helper cell imbalance towards the Th1 subtype. |

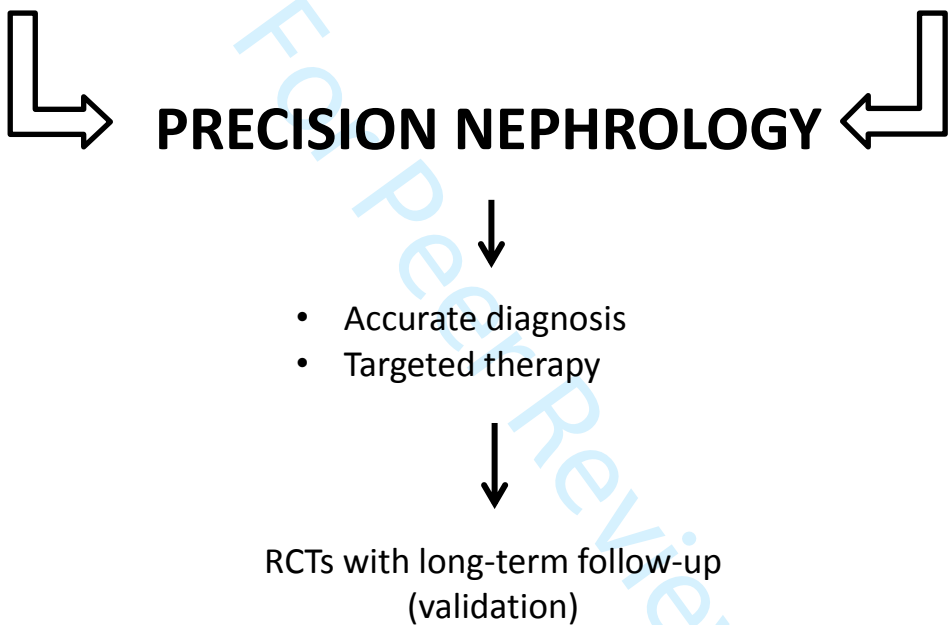
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|---|---|--|----------------------------|--|
| 2 | Production of anti-Gd-IgA1 autoantibodies of IgG and/or IgA isotype | High Gd-IgA1 elicits an autoimmune response, resulting in generation of anti-glycan antibodies that recognize N-acetylgalactosamine epitopes on Gd-IgA1 | common genetic variants | Anti-glycan response may be triggered by the exposure to infectious or dietary antigens that have been aberrantly processed by genetically predisposing MHC class II allele loci: <i>HLA-DQA1</i> , <i>HLA-DQB1</i> , <i>HLA-DRB1</i> , <i>HLA-DP</i> having a role in autoimmunity |
| | | | gene expression studies | Defect in antigen handling in PBMCs with an up-regulation of interferon signalling and immunoproteasome pathway (PSMB8, PSMB9, PSMB10, TAPBP) has been demonstrated in IgAN patients. These pathways seem to be upregulated in autoimmune diseases |
| 3 | Formation of circulating IgA1–IgG and IgA1–IgA1 immune complexes and deposition | Immune complexes activate alternative complement pathway | common genetic variants | ITGAM is essential for interactions between CD89 and secretory IgA |
| | | | gene expression studies | -Hyperactivation of PI3K pathway in monocytes activates FcαRI and circulating immune complexes aggregate this receptor. This interaction induces shedding of the extracellular domain to form circulating IgA1–FcαRI complexes that could have a pathogenic role in IgAN. - Gene expression studies on kidney tissue have evidenced ITGAM |
| 4 | Local activation of inflammatory pathways and the complement system | local inflammation, mesangial cell proliferation, secretion pro-inflammatory cytokines leading to the interstitial infiltration of inflammatory cells and promoting glomerular and tubulointerstitial fibrosis | common genetic variants | - Glomerular inflammation is enhanced by the complement system - lectin or the alternative pathway (CFHR3,1-del, CFHR1 and CFHR3) - ITGAM and ITGAX encode integrins that combine to form leukocyte specific complement receptor CR3 and CR4. These may be involved in glomerular inflammation. |
| | | | gene expression studies | -Various gene expression studies conducted on kidney tissue show the involvement of relevant signalling pathways: Activation Complement system (<i>ITGAX-ITGAM</i> , <i>C1QA</i> , <i>C1QB</i>); Toll-like receptor Signalling; NF-κB pathway; IL-8 signalling; Production of nitric oxide and reactive oxygen species in macrophages; -Kidney production of cytokines (mainly IL6) promotes mesangial cell proliferation |
| | | | miRNA & metilation studies | -TLR9 CNV contributes to the deterioration of the renal function |
| | | | Proteomics | -Low levels of UMOD peptides cause progression of renal damage |

CLINICAL FINDINGS

Phenotypic pattern (micro/macro-hematuria proteinuria)
eGFR
Proteinuria
Kidney biopsy (Damage score)

MOLECULAR DATA

Genomics (CVs, RVs)
Transcriptomics (mRNA)
Epigenomics (Methyl DNA regions, miRNAs)
Proteomics (polypeptides)
Metabolomics (Metabolites)



CVs: common variants; ; **RVs:** rare variants; **mRNA:**messenger RNA; **miRNAs:** microRNA; **eGFR:**Estimated Glomerular Filtration Rate; **RCTs:**Randomized Controlled Trials;